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PCT/GB 00/03556



GB00/3536



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REC'D 05 OCT 2000	
The Patent Office	PCT
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Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

10/088854

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

I also certify that by virtue of an assignment registered under the Patents Act 1977, the application is now proceeding in the name as substituted.

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Andrew Gersey

Dated 21 September 2000

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GB9922152.5

By virtue of a direction given under Section of the Patents Act 1977, the application is proceeding in the name of

ASTRAZENECA AB,
Incorporated in Sweden,
S-151 85 Sodertalje,
Sweden

[ADP No. 07822448003]

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GB9922152.5

By virtue of a direction given under Section of the Patents Act 1977, the application is proceeding in the name of

ASTRAZENECA UK LIMITED
Incorporated in the United Kingdom
15 Stanhope Gate
LONDON
W1Y 0AN
United Kingdom

[ADP No. 07810294001]

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21 SEP 1999

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The Patent Office

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The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference PHM 99-145

2. Patent application number
(The Patent Office will fill in this part)

9922152.5

21 SEP 1999

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Zeneca Limited
15 Stanhope Gate
LONDON

SECTION 30 (1977 ACT) APPLICATION FILED 9/3/2000

Patents ADP number (if you know it)

6254007002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

CHEMICAL COMPOUNDS

5. Name of your agent (if you have one)

BILL, Kevin

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

AstraZeneca PLC
Global Intellectual Property
Mereside, Alderley Park,
Macclesfield, Cheshire, SK10 4TG, GB

Patents ADP number (if you know it)

4469847002

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Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

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- b) there is an inventor who is not named as an applicant, or

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Description

29

Claim(s)

Abstract

Drawing(s)

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Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature

Date

Lynda May Slack

20 September 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

Mrs Lynda May Slack 01625 516173

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CHEMICAL COMPOUNDS

The present invention relates to certain quinazoline derivatives for use in the treatment of certain diseases in particular to proliferative disease such as cancer and in the preparation of medicaments for use in the treatment of proliferative disease, to novel
5 quinazoline compounds and to processes for their preparation, as well as pharmaceutical compositions containing them as active ingredient.

Cancer (and other hyperproliferative disease) is characterised by uncontrolled cellular proliferation. This loss of the normal regulation of cell proliferation often appears to occur as
10 the result of genetic damage to cellular pathways that control progress through the cell cycle.

In eukaryotes, the cell cycle is largely controlled by an ordered cascade of protein phosphorylation. Several families of protein kinases that play critical roles in this cascade have now been identified. The activity of many of these kinases is increased in human tumours when compared to normal tissue. This can occur by either increased levels of
15 expression of the protein (as a result of gene amplification for example), or by changes in expression of co activators or inhibitory proteins.

The first identified, and most widely studied of these cell cycle regulators have been the cyclin dependent kinases (or CDKs). Activity of specific CDKs at specific times is essential for both initiation and coordinated progress through the cell cycle. For example, the
20 CDK4 protein appears to control entry into the cell cycle (the G0-G1-S transition) by phosphorylating the retinoblastoma gene product pRb. This stimulates the release of the transcription factor E2F from pRb, which then acts to increase the transcription of genes necessary for entry into S phase. The catalytic activity of CDK4 is stimulated by binding to a partner protein, Cyclin D. One of the first demonstrations of a direct link between cancer and
25 the cell cycle was made with the observation that the Cyclin D1 gene was amplified and cyclin D protein levels increased (and hence the activity of CDK4 increased) in many human tumours (Reviewed in Sherr, 1996, Science 274: 1672-1677; Pines, 1995, Seminars in Cancer Biology 6: 63-72). Other studies (Loda et al., 1997, Nature Medicine 3(2): 231-234; Gemma et al., 1996, International Journal of Cancer 68(5): 605-11; Elledge et al. 1996, Trends in Cell
30 Biology 6; 388-392) have shown that negative regulators of CDK function are frequently

down regulated or deleted in human tumours again leading to inappropriate activation of these kinases.

More recently, protein kinases that are structurally distinct from the CDK family have been identified which play critical roles in regulating the cell cycle and which also appear to be important in oncogenesis. These include the newly identified human homologues of the

~~*Drosophila* aurora and *S.cerevisiae* Ipl1 proteins. *Drosophila* aurora and *S.cerevisiae* Ipl1,~~

which are highly homologous at the amino acid sequence level, encode serine/threonine protein kinases. Both aurora and Ipl1 are known to be involved in controlling the transition from the G2 phase of the cell cycle through mitosis, centrosome function, formation of a mitotic spindle and proper chromosome separation / segregation into daughter cells. The two human homologues of these genes, termed aurora1 and aurora2, encode cell cycle regulated protein kinases. These show a peak of expression and kinase activity at the G2/M boundary (aurora2) and in mitosis itself (aurora1). Several observations implicate the involvement of human aurora proteins, and particularly aurora2 in cancer. The aurora2 gene maps to chromosome 20q13, a region that is frequently amplified in human tumours including both breast and colon tumours. Aurora2 may be the major target gene of this amplicon, since aurora2 DNA is amplified and aurora2 mRNA overexpressed in greater than 50% of primary human colorectal cancers. In these tumours aurora2 protein levels appear greatly elevated compared to adjacent normal tissue. In addition, transfection of rodent fibroblasts with human aurora2 leads to transformation, conferring the ability to grow in soft agar and form tumours in nude mice (Bischoff et al., 1998, The EMBO Journal. 17(11): 3052-3065). Other work (Zhou et al., 1998, Nature Genetics. 20(2): 189-93) has shown that artificial overexpression of aurora2 leads to an increase in centrosome number and an increase in aneuploidy.

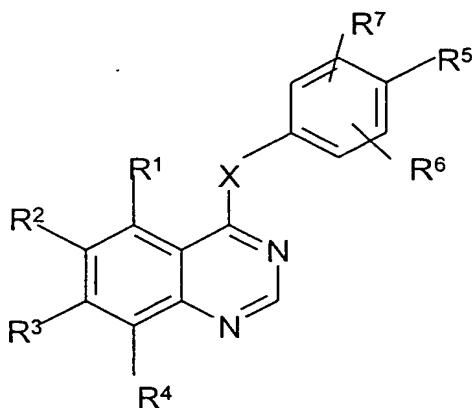
Importantly, it has also been demonstrated that abrogation of aurora2 expression and function by antisense oligonucleotide treatment of human tumour cell lines (WO 97/22702 and WO 99/37788) leads to cell cycle arrest in the G2 phase of the cell cycle and exerts an antiproliferative effect in these tumour cell lines. This indicates that inhibition of the function of aurora2 will have an antiproliferative effect that may be useful in the treatment of human tumours and other hyperproliferative diseases.

A number of quinazoline derivatives have been proposed hitherto for use in the inhibition of various kinases. For example, WO 96/09294, WO 96/15118, WO 99/06378,

WO 96/30347, US Patent No 5646155, EP-A-566226 amongst others, describe the use of certain quinazoline compounds as receptor tyrosine kinase inhibitors, which may be useful in the treatment of proliferative disease.

The applicants have found a series of compounds which inhibit the effect of the aurora2 kinase and which are thus of use in the treatment of proliferative disease such as cancer, in particular in such diseases such as colorectal or breast cancer where aurora 2 kinase is known to be active.

The present invention provides the use of a compound of formula (I)



(I)

or a salt, ester or , amide thereof;

where X is O, or S, S(O) or S(O)₂, NH or NR⁸ where R⁸ is hydrogen or C₁₋₆alkyl;

R⁵ is an optionally substituted hydrocarbonyl group (other than ethenyl substituted by a carboxy group or an amide or sulphonamide derivative thereof),

R⁶ and R⁷ are independently selected from hydrogen, halo, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkoxymethyl, di(C₁₋₄alkoxy)methyl, C₁₋₄alkanoyl, trifluoromethyl, cyano, amino, C₂₋₃alkenyl, C₂₋₃alkynyl, a phenyl group, a benzyl group or a 5-6-membered heterocyclic group with 1-3 heteroatoms, selected independently from O, S and N, which heterocyclic group may be aromatic or non-aromatic and may be saturated (linked via a ring carbon or nitrogen atom) or unsaturated (linked via a ring carbon atom), and which phenyl, benzyl or heterocyclic group may bear on one or more ring carbon atoms up to 5 substituents selected from hydroxy, halogeno, C₁₋₃alkyl, C₁₋₃alkoxy, C₁₋₃alkanoyloxy, trifluoromethyl, cyano, amino, nitro, C₂₋

- C_{1-4} alkanoyl, C_{1-4} alkanoylamino, C_{1-4} alkoxycarbonyl, C_{1-4} alkylsulphanyl, C_{1-4} alkylsulphinyl, C_{1-4} alkylsulphonyl, carbamoyl, N - C_{1-4} alkylcarbamoyl, N,N -di(C_{1-4} alkyl)carbamoyl, aminosulphonyl, N - C_{1-4} alkylaminosulphonyl, N,N -di(C_{1-4} alkyl)aminosulphonyl, C_{1-4} alkylsulphonylamino, and a saturated heterocyclic group selected from morpholino, thiomorpholino, pyrrolidinyl, piperazinyl, piperidinyl imidazolidinyl and pyrazolidinyl, which
-
- ~~saturated heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-3} alkyl, C_{1-3} alkoxy, C_{1-3} alkanoyloxy, trifluoromethyl, cyano, amino, nitro and C_{1-4} alkoxycarbonyl, and~~
- R^1, R^2, R^3, R^4 are independently selected from, halo, cyano, nitro, trifluoromethyl, C_{1-3} alkyl, $-NR^9R^{10}$ (wherein R^9 and R^{10} , which may be the same or different, each represents hydrogen or C_{1-3} alkyl), or $-X^1R^{11}$ (wherein X^1 represents a direct bond; $-O-$, $-CH_2-$, $-OCO-$, carbonyl, $-S-$, $-SO-$, $-SO_2-$, $-NR^{12}CO-$, $-CONR^{12}-$, $-SO_2NR^{12}-$, $-NR^{13}SO_2-$ or $-NR^{14}-$ (wherein R^{12}, R^{13} and R^{14} each independently represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl), and R^{11} is selected from one of the following eighteen groups:
- 1) hydrogen or C_{1-3} alkyl which may be unsubstituted or which may be substituted with one or more groups selected from hydroxy, fluoro and amino;
 - 2) $C_{1-3}alkylX^2COR^{15}$ (wherein X^2 represents $-O-$ or $-NR^{16}-$ (in which R^{15} represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{16} represents C_{1-3} alkyl, $-NR^{17}R^{18}$ or $-OR^{19}$ (wherein R^{17}, R^{18} and R^{19} which may be the same or different each represents hydrogen, C_{1-3} alkyl or $C_{1-3}alkoxyC_{2-3}alkyl$));
 - 3) $C_{1-3}alkylX^3R^{20}$ (wherein X^3 represents $-O-$, $-S-$, $-SO-$, $-SO_2-$, $-OCO-$, $-NR^{21}CO-$, $-CONR^{22}-$, $-SO_2NR^{23}-$, $-NR^{24}SO_2-$ or $-NR^{25}-$ (wherein $R^{21}, R^{22}, R^{23}, R^{24}$ and R^{25} each independently represents hydrogen, C_{1-3} alkyl or $C_{1-3}alkoxyC_{2-3}alkyl$) and R^{20} represents hydrogen, C_{1-3} alkyl, cyclopentyl, cyclohexyl or a 5-6-membered saturated heterocyclic group with 1-2 heteroatoms, selected independently from O, S and N, which C_{1-3} alkyl group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno and C_{1-4} alkoxy and which cyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4} alkyl, C_{1-4} hydroxyalkyl and C_{1-4} alkoxy);
 - 4) $C_{1-3}alkylX^4C_{1-3}alkylX^5R^{26}$ (wherein X^4 and X^5 which may be the same or different are each $-O-$, $-S-$, $-SO-$, $-SO_2-$, $-NR^{27}CO-$, $-CONR^{28}-$, $-SO_2NR^{29}-$, $-NR^{30}SO_2-$ or $-NR^{31}-$ (wherein $R^{27}, R^{28},$

R^{29} , R^{30} and R^{31} each independently represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{26} represents hydrogen or C_{1-3} alkyl);

5) R^{32} (wherein R^{32} is a 5-6-membered saturated heterocyclic group (linked via carbon or nitrogen) with 1-2 heteroatoms, selected independently from O, S and N, which heterocyclic group may bear 1 or 2 substituents selected from oxo, hydroxy, halogeno, C_{1-4} alkyl, C_{1-4} hydroxyalkyl, C_{1-4} alkoxy, C_{1-4} alkoxy C_{1-4} alkyl and C_{1-4} alkylsulphonyl C_{1-4} alkyl),

6) C_{1-5} alkyl R^{32} (wherein R^{32} is as defined hereinbefore);

7) C_{2-5} alkenyl R^{32} (wherein R^{32} is as defined hereinbefore);

8) C_{2-5} alkynyl R^{32} (wherein R^{32} is as defined hereinbefore);

9) R^{33} (wherein R^{33} represents a pyridone group, a phenyl group or a 5-6-membered aromatic heterocyclic group (linked via carbon or nitrogen) with 1-3 heteroatoms selected from O, N and S, which pyridone, phenyl or aromatic heterocyclic group may carry up to 5 substituents on an available carbon atom selected from hydroxy, halogeno, amino, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} aminoalkyl, C_{1-4} alkylamino, C_{1-4} hydroxyalkoxy, carboxy,

trifluoromethyl, cyano, $-\text{CONR}^{34}\text{R}^{35}$ and $-\text{NR}^{36}\text{COR}^{37}$ (wherein R^{34} , R^{35} , R^{36} and R^{37} , which may be the same or different, each represents hydrogen, C_{1-4} alkyl or C_{1-3} alkoxy C_{2-3} alkyl));

10) C_{1-5} alkyl R^{33} (wherein R^{33} is as defined hereinbefore);

11) C_{2-5} alkenyl R^{33} (wherein R^{33} is as defined hereinbefore);

12) C_{2-5} alkynyl R^{33} (wherein R^{33} is as defined hereinbefore);

13) C_{1-5} alkyl X^6R^{33} (wherein X^6 represents $-\text{O}-$, $-\text{S}-$, $-\text{SO}-$, $-\text{SO}_2-$, $-\text{NR}^{38}\text{CO}-$, $-\text{CONR}^{39}-$, $-\text{SO}_2\text{NR}^{40}-$, $-\text{NR}^{41}\text{SO}_2-$ or $-\text{NR}^{42}-$ (wherein R^{38} , R^{39} , R^{40} , R^{41} and R^{42} each independently represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{33} is as defined hereinbefore);

14) C_{2-5} alkenyl X^7R^{33} (wherein X^7 represents $-\text{O}-$, $-\text{S}-$, $-\text{SO}-$, $-\text{SO}_2-$, $-\text{NR}^{43}\text{CO}-$, $-\text{CONR}^{44}-$, $-\text{SO}_2\text{NR}^{45}-$, $-\text{NR}^{46}\text{SO}_2-$ or $-\text{NR}^{47}-$ (wherein R^{43} , R^{44} , R^{45} , R^{46} and R^{47} each independently represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{33} is as defined hereinbefore);

15) C_{2-5} alkynyl X^8R^{33} (wherein X^8 represents $-\text{O}-$, $-\text{S}-$, $-\text{SO}-$, $-\text{SO}_2-$, $-\text{NR}^{48}\text{CO}-$, $-\text{CONR}^{49}-$, $-\text{SO}_2\text{NR}^{50}-$, $-\text{NR}^{51}\text{SO}_2-$ or $-\text{NR}^{52}-$ (wherein R^{48} , R^{49} , R^{50} , R^{51} and R^{52} each independently represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{33} is as defined hereinbefore);

16) C_{1-3} alkyl X^9C_{1-3} alkyl R^{33} (wherein X^9 represents $-\text{O}-$, $-\text{S}-$, $-\text{SO}-$, $-\text{SO}_2-$, $-\text{NR}^{53}\text{CO}-$, $-\text{CONR}^{54}-$, $-\text{SO}_2\text{NR}^{55}-$, $-\text{NR}^{56}\text{SO}_2-$ or $-\text{NR}^{57}-$ (wherein R^{53} , R^{54} , R^{55} , R^{56} and R^{57} each independently represents hydrogen, C_{1-3} alkyl or C_{1-3} alkoxy C_{2-3} alkyl) and R^{33} is as defined hereinbefore); and

17) $C_{1-3}alkylX^9C_{1-3}alkylR^{32}$ (wherein X^9 and R^{32} are as defined hereinbefore);
 and R^1 and R^4 may additionally be hydrogen; in the preparation of a medicament for use in
 the inhibition of aurora 2 kinase. In particular, such medicaments are useful in the treatment of
 proliferative disease such as cancer, and in particular cancers where aurora 2 is upregulated
 5 such as colon or breast cancers.

~~In this specification the term 'alkyl' when used either alone or as a suffix includes~~
 straight chained, branched structures. Unless otherwise stated, these groups may contain up to
 10, preferably up to 6 and more preferably up to 4 carbon atoms. Similarly the terms
 "alkenyl" and "alkynyl" refer to unsaturated straight or branched structures containing for
 10 example from 2 to 10, preferably from 2 to 6 carbon atoms. Cyclic moieties such as
 cycloalkyl, cycloalkenyl and cycloalkynyl are similar in nature but have at least 3 carbon
 atoms. Terms such as "alkoxy" comprise alkyl groups as is understood in the art.

The term "halo" includes fluoro, chloro, bromo and iodo. References to aryl groups
 include aromatic carbocyclic groups such as phenyl and naphthyl. The term "heterocyclyl"
 15 includes aromatic or non-aromatic rings, for example containing from 4 to 20, suitably from 5
 to 8 ring atoms, at least one of which is a heteroatom such as oxygen, sulphur or nitrogen.
 Examples of such groups include furyl, thienyl, pyrrolyl, pyrrolidinyl, imidazolyl, triazolyl,
 thiazolyl, tetrazolyl, oxazolyl, isoxazolyl, pyrazolyl, pyridyl, pyrimidinyl, pyrazinyl,
 pyridazinyl, triazinyl, quinolinyl, isoquinolinyl, quinoxalinyl, benzothiazolyl, benzoxazolyl,
 20 benzothienyl or benzofuryl.

"Heteroaryl" refers to those groups described above which have an aromatic character.
 The term "aralkyl" refers to aryl substituted alkyl groups such as benzyl.

Other expressions used in the specification include "hydrocarbyl" which refers to any
 structure comprising carbon and hydrogen atoms. For example, these may be alkyl, alkenyl,
 25 alkynyl, aryl, heterocyclyl, alkoxy, aralkyl, cycloalkyl, cycloalkenyl or cycloalkynyl.

The term "functional group" refers to reactive substituents such as nitro, cyano, halo,
 oxo, $=CR^{78}R^{79}$, $C(O)_xR^{77}$, OR^{77} , $S(O)_yR^{77}$, $NR^{78}R^{79}$, $C(O)NR^{78}R^{79}$, $OC(O)NR^{78}R^{79}$, $=NOR^{77}$, $-$
 $NR^{77}C(O)_xR^{78}$, $-NR^{77}CONR^{78}R^{79}$, $-N=CR^{78}R^{79}$, $S(O)_yNR^{78}R^{79}$ or $-NR^{77}S(O)_yR^{78}$ where R^{77} ,
 R^{78} and R^{79} are independently selected from hydrogen or optionally substituted hydrocarbyl,
 30 or R^{78} and R^{79} together form an optionally substituted ring which optionally contains further

heteroatoms such as $S(O)_y$, oxygen and nitrogen, x is an integer of 1 or 2, y is 0 or an integer of 1-3.

Suitable optional substituents for hydrocarbonyl groups R^{77} , R^{78} and R^{79} include halo, perhaloalkyl such as trifluoromethyl, mercapto, hydroxy, carboxy, alkoxy, heteroaryl, heteroaryloxy, alkenyloxy, alkynyloxy, alkoxyalkoxy, aryloxy (where the aryl group may be substituted by halo, nitro, or hydroxy), cyano, nitro, amino, mono- or di-alkyl amino, oximino or $S(O)_y$, where y is as defined above.

Preferably R^1 and R^4 are hydrogen.

In a preferred embodiment, at least one group R^2 or R^3 , preferably R^3 , comprises a chain of at least 3 and preferably at least 4 optionally substituted carbon atoms or heteroatoms such as oxygen, nitrogen or sulphur. Most preferably the chain is substituted by a polar group which assists in solubility.

Suitably R^3 is a group XR^{11} . Preferably in this case, X^1 is oxygen and R^{11} is selected from a group of formula (1) or (10) above. Particular groups R^{11} are those in group (1) above, especially alkyl such as methyl or halo substituted alkyl, or those in group (10) above. In one preferred embodiment, at least one of R^2 or R^3 is a group $OC_{1-3}alkylR^{33}$ and R^{33} is a heterocyclic ring such as an N-linked morpholine ring such as 3-morpholinopropoxy.

Suitably R^2 is selected from, halo, cyano, nitro, trifluoromethyl, $C_{1-3}alkyl$, $-NR^9R^{10}$ (wherein R^9 and R^{10} , which may be the same or different, each represents hydrogen or $C_{1-3}alkyl$), or a group $-X^1R^{11}$. Preferred examples of $-X^1R^{11}$ for R^2 include those listed above in relation to R^3 .

Other examples for R^2 and R^3 include methoxy or 3,3,3-trifluoroethoxy.

Preferably X is NH or O and is most preferably NH.

Examples of R^5 include $C_{1-6}alkyl$, optionally substituted by a functional group, in particular by carboxy or an $C_{1-6}alkyl$ ester thereof, or cyano, or by an aryl group such as phenyl which may itself be substituted by a functional group. A particular example of R^5 is benzyl and cyanobenzyl.

Another example of R^5 is optionally substituted aryl such as phenyl, where the optional substituents include $C_{1-3}alkyl$ groups as well as functional groups in particular nitro and halo such as bromo,

Further examples of R^5 groups include C_{2-6} alkynyl in particular ethynyl, which may be optionally substituted for example with trimethylsilyl groups.

Suitably R^6 and R^7 are independently selected from hydrogen halo, C_{1-4} alkoxy such as methoxy, or ethoxy, cyano, trifluoromethyl, or phenyl.

5 Preferably R^6 and R^7 are hydrogen.

~~Suitable pharmaceutically acceptable salts of compounds of formula (I) include acid~~
addition salts such as methanesulfonate, fumarate, hydrochloride, hydrobromide, citrate, maleate and salts formed with phosphoric and sulphuric acid. There may be more than one cation or anion depending on the number of charged functions and the valency of the cations or anions. Where the compound of formula (I) includes an acid functionality, salts may be
10 base salts such as an alkali metal salt for example sodium, an alkaline earth metal salt for example calcium or magnesium, an organic amine salt for example triethylamine, morpholine, *N*-methylpiperidine, *N*-ethylpiperidine, procaine, dibenzylamine, *N,N*-dibenzylethylamine or amino acids for example lysine. A preferred pharmaceutically acceptable salt is a sodium salt.

15 An *in vivo* hydrolysable ester of a compound of the formula (I) containing carboxy or hydroxy group is, for example, a pharmaceutically acceptable ester which is hydrolysed in the human or animal body to produce the parent acid or alcohol.

Suitable pharmaceutically acceptable esters for carboxy include C_{1-6} alkyl esters such as methyl or ethyl esters, C_{1-6} alkoxymethyl esters for example methoxymethyl,
20 C_{1-6} alkanoyloxymethyl esters for example pivaloyloxymethyl, phthalidyl esters, C_{3-8} cycloalkoxy-carbonyloxy C_{1-6} alkyl esters for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolen-2-onylmethyl esters for example 5-methyl-1,3-dioxolen-2-onylmethyl; and C_{1-6} alkoxycarbonyloxyethyl esters for example 1-methoxycarbonyloxyethyl and may be formed at any carboxy group in the compounds of this invention.

25 An *in vivo* hydrolysable ester of a compound of the formula (I) containing a hydroxy group includes inorganic esters such as phosphate esters and α -acyloxyalkyl ethers and related compounds which as a result of the *in vivo* hydrolysis of the ester breakdown to give the parent hydroxy group. Examples of α -acyloxyalkyl ethers include acetoxymethoxy and 2,2-dimethylpropionyloxymethoxy. A selection of *in vivo* hydrolysable ester forming groups
30 for hydroxy include alkanoyl, benzoyl, phenylacetyl and substituted benzoyl and phenylacetyl, alkoxycarbonyl (to give alkyl carbonate esters), dialkylcarbamoyl and

N-(dialkylaminoethyl)-*N*-alkylcarbamoyl (to give carbamates), dialkylaminoacetyl and carboxyacetyl.

Suitable amides are derived from compounds of formula (I) which have a carboxy group which is derivatised into an amide such as a *N*-C₁₋₆alkyl and *N,N*-di-

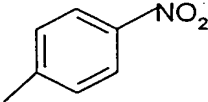
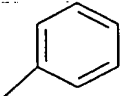
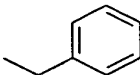
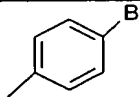
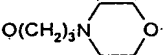
- 5 (C₁₋₆alkyl)amide such as *N*-methyl, *N*-ethyl, *N*-propyl, *N,N*-dimethyl, *N*-ethyl-*N*-methyl or *N,N*-diethylamide.

Esters which are not *in vivo* hydrolysable may be useful as intermediates in the production of the compounds of formula (I).

Particular examples of compounds of formula (I) are set out in Table 1

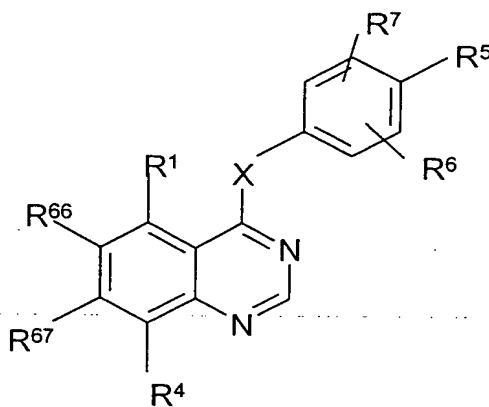
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Table 1

Comp No	R ²	R ³	R ⁵	R ⁶	R ⁷	X
1	OCH ₃	OCH ₃	(CH ₂) ₂ CO ₂ CH ₃	H	H	O
2	OCH ₃	OCH ₃		H	H	O
3	OCH ₃	OCH ₃		H	H	O
4	OCH ₃	OCH ₃	(CH ₂) ₂ CH ₃	H	H	O
5	OCH ₃	OCH ₃		H	H	O
6	OCH ₃	OCH ₃		H	H	O
7	OCH ₃	OCH ₃	CH ₂ CH ₂ CN	H	H	O
8	OCH ₃	OCH ₃	C≡CSi(CH ₃) ₂	H	H	NH
9	OCH ₃	OCH ₃	C≡CH	H	H	NH
10	OCH ₃	OCH ₃	(CH ₂) ₂ CO ₂ CH ₂ CH ₃	H	H	NH
11	OCH ₃		CH(CN)C ₆ H ₅	H	H	NH

12	OCH ₃		(CH ₂) ₅ CH ₃	H	H	NH
13	OCH ₃		(CH ₂) ₃ CH ₃	H	H	NH
14	OCH ₃		CH ₂ C ₆ H ₅	H	H	NH

Certain compounds of formula (I) are novel and these form a further aspect of the invention. Examples of such compounds include a compound of formula (IA)



(IA)

where X, Y, R¹, R², R⁵, R⁶ and R⁷ and n are as defined in relation to formula (I) and R⁶⁶ is halo, cyano, nitro, trifluoromethyl, C₁₋₃alkyl, -NR⁹R¹⁰ (wherein R⁹ and R¹⁰, which may be the same or different, each represents hydrogen or C₁₋₃alkyl), or a group -X¹R¹¹ where X¹ and R¹¹ are as defined in relation to formula (I) and R¹¹ is particularly a group of sub group (1) or (10), and R⁶⁷ is C₁₋₆alkoxy optionally substituted by fluorine or a group X¹R³³ in which X¹ and R³³ are as defined in relation to formula (I), and in particular X¹ is oxygen and R³³ is or a 5-6-membered aromatic heterocyclic group (linked via nitrogen) with 1-3 heteroatoms selected from O, N and S; provided that at least one of R⁶⁶ and R⁶⁷ is other than unsubstituted methoxy.

A preferred example of R⁶⁷ is 3-morpholinopropoxy.

Preferably X¹ is oxygen.

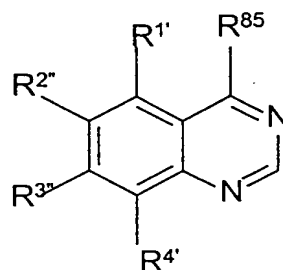
Preferably at least R^{67} is other than unsubstituted alkoxy.

Where R^{66} or R^{67} is unsubstituted alkoxy, it is preferably methoxy.

Suitable halo substituents for R^{66} and R^{67} are fluoro.

Other examples for R^{66} and/or R^{67} include 3,3,3-trifluoroethoxy.

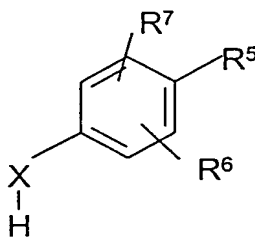
5 Compounds of formula (I) may be prepared by methods known in the art or by analogous methods. For example, a compound of formula (I) can be prepared by reacting a compound of formula (II)



(II)

10

where $R^{1'}$, $R^{2''}$, $R^{3''}$, and $R^{4'}$ are equivalent to a group R^1 , R^2 , R^3 and R^4 as defined in relation to formula (I) or a precursor thereof, and R^{85} is a leaving group, with a compound of formula (VIII)



(III)

15

where X , R^5 , R^6 , R^7 and n are as defined in relation to formula (I), and thereafter if desired or necessary converting a group $R^{1'}$, $R^{2''}$, $R^{3''}$ or $R^{4'}$ to a group R^1 , R^2 , R^3 and R^4 respectively or to a different such group.

Suitable leaving groups for R^{85} include halo such as chloro, mesylate and tosylate.

20 The reaction is suitably effected in an organic solvent such as an alcohol like isopropanol, at elevated temperatures, conveniently at the reflux temperature of the solvent.

The conversion of a group R^{1'}, R^{2'}, R^{3'} or R^{4'} to a group R¹, R², R³ and R⁴ respectively or to a different such group, may be particularly useful in connection with the preparation of compounds of formula (IA) and examples of these preparations are provided hereinafter.

Compounds of formula (II) and (III) are either known compounds or they can be
5 derived from known compounds by conventional methods.

~~Compounds of formula (I) are inhibitors of aurora 2 kinase. As a result, these~~
compounds can be used to treat disease mediated by these agents, in particular proliferative disease.

According to a further aspect of the present invention there is provided a method for
10 inhibiting aurora 2 kinase in a warm blooded animal, such as man, in need of such treatment, which comprises administering to said animal an effective amount of a compound of formula (I), or a pharmaceutically acceptable salt, or an *in vivo* hydrolysable ester thereof.

Novel compounds of formula (I) have not hitherto been proposed for use in therapy. Thus, according to a further aspect of the invention there is provided a compound of the
15 formula (IA) as defined herein, or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof, for use in a method of treatment of the human or animal body by therapy. In particular, the compounds are used in methods of treatment of proliferative disease such as cancer and in particular cancers such as colorectal or breast cancer where aurora 2 is upregulated.

Compounds of formula (I) are suitably applied in the form of a pharmaceutical
20 composition. Preferred compounds of formula (I) for use in the compositions of the invention are as described above.

Some of these are novel and form yet a further aspect of the invention. Thus, the invention also provides a pharmaceutical composition comprising a novel compound of
25 formula (I) such as a compound of formula (IA) as defined herein, or a pharmaceutically acceptable salt, or an *in vivo* hydrolysable ester thereof, in combination with at pharmaceutically acceptable carrier.

The compositions of compounds of formula (I) may be in a form suitable for oral use
(for example as tablets, lozenges, hard or soft capsules, aqueous or oily suspensions,
30 emulsions, dispersible powders or granules, syrups or elixirs), for topical use (for example as creams, ointments, gels, or aqueous or oily solutions or suspensions), for administration by

inhalation (for example as a finely divided powder or a liquid aerosol), for administration by insufflation (for example as a finely divided powder) or for parenteral administration (for example as a sterile aqueous or oily solution for intravenous, subcutaneous, intramuscular or intramuscular dosing or as a suppository for rectal dosing).

5 The compositions of the invention may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art. Thus, compositions intended for oral use may contain, for example, one or more colouring, sweetening, flavouring and/or preservative agents.

10 Suitable pharmaceutically acceptable excipients for a tablet formulation include, for example, inert diluents such as lactose, sodium carbonate, calcium phosphate or calcium carbonate, granulating and disintegrating agents such as corn starch or algenic acid; binding agents such as starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl p-hydroxybenzoate, and anti-oxidants, such as ascorbic acid. Tablet formulations may be uncoated or coated either to modify their
15 disintegration and the subsequent absorption of the active ingredient within the gastrointestinal track, or to improve their stability and/or appearance, in either case, using conventional coating agents and procedures well known in the art.

 Compositions for oral use may be in the form of hard gelatin capsules in which the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium
20 phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin, or olive oil.

 Aqueous suspensions generally contain the active ingredient in finely powdered form together with one or more suspending agents, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum
25 tragacanth and gum acacia; dispersing or wetting agents such as lecithin or condensation products of an alkylene oxide with fatty acids (for example polyoxyethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or
30 condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters

derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives (such as ethyl or propyl p-hydroxybenzoate, anti-oxidants (such as ascorbic acid), colouring agents, flavouring agents, and/or sweetening agents (such as sucrose, saccharine or aspartame).

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil (such as arachis oil, olive oil, sesame oil or coconut oil) or in a mineral oil (such as liquid paraffin). The oily suspensions may also contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set out above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water generally contain the active ingredient together with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients such as sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, or a mineral oil, such as for example liquid paraffin or a mixture of any of these. Suitable emulsifying agents may be, for example, naturally-occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soya bean, lecithin, an esters or partial esters derived from fatty acids and hexitol anhydrides (for example sorbitan monooleate) and condensation products of the said partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring and preservative agents.

Syrups and elixirs may be formulated with sweetening agents such as glycerol, propylene glycol, sorbitol, aspartame or sucrose, and may also contain a demulcent, preservative, flavouring and/or colouring agent.

The pharmaceutical compositions may also be in the form of a sterile injectable aqueous or oily suspension, which may be formulated according to known procedures using

one or more of the appropriate dispersing or wetting agents and suspending agents, which have been mentioned above. A sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example a solution in 1,3-butanediol.

5 Suppository formulations may be prepared by mixing the active ingredient with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Suitable excipients include, for example, cocoa butter and polyethylene glycols.

10 Topical formulations, such as creams, ointments, gels and aqueous or oily solutions or suspensions, may generally be obtained by formulating an active ingredient with a conventional, topically acceptable, vehicle or diluent using conventional procedure well known in the art.

15 Compositions for administration by insufflation may be in the form of a finely divided powder containing particles of average diameter of, for example, 30 μ or much less, the powder itself comprising either active ingredient alone or diluted with one or more physiologically acceptable carriers such as lactose. The powder for insufflation is then conveniently retained in a capsule containing, for example, 1 to 50mg of active ingredient for use with a turbo-inhaler device, such as is used for insufflation of the known agent sodium cromoglycate.

20 Compositions for administration by inhalation may be in the form of a conventional pressurised aerosol arranged to dispense the active ingredient either as an aerosol containing finely divided solid or liquid droplets. Conventional aerosol propellants such as volatile fluorinated hydrocarbons or hydrocarbons may be used and the aerosol device is conveniently arranged to dispense a metered quantity of active ingredient.

25 For further information on Formulation the reader is referred to Chapter 25.2 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

30 The amount of active ingredient that is combined with one or more excipients to produce a single dosage form will necessarily vary depending upon the host treated and the particular route of administration. For example, a formulation intended for oral administration to humans will generally contain, for example, from 0.5 mg to 2 g of active agent

compounded with an appropriate and convenient amount of excipients which may vary from about 5 to about 98 percent by weight of the total composition. Dosage unit forms will generally contain about 1 mg to about 500 mg of an active ingredient. For further information on Routes of Administration and Dosage Regimes the reader is referred to Chapter 25.3 in
5 Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The size of the dose for therapeutic or prophylactic purposes of a compound of the Formula I will naturally vary according to the nature and severity of the conditions, the age and sex of the animal or patient and the route of administration, according to well known
10 principles of medicine. As mentioned above, compounds of the Formula I are useful in treating diseases or medical conditions which are due alone or in part to the effects of aurora 2 kinase.

In using a compound of the Formula I for therapeutic or prophylactic purposes it will generally be administered so that a daily dose in the range, for example, 0.5 mg to 75 mg per
15 kg body weight is received, given if required in divided doses. In general lower doses will be administered when a parenteral route is employed. Thus, for example, for intravenous administration, a dose in the range, for example, 0.5 mg to 30 mg per kg body weight will generally be used. Similarly, for administration by inhalation, a dose in the range, for example, 0.5 mg to 25 mg per kg body weight will be used. Oral administration is however
20 preferred.

The following Examples illustrate the invention.

Example 1 - Preparation of Compound No. 1 in Table 1

4-Chloro-6,7-dimethoxyquinazoline (112 mg, 0.50 mmol) and potassium carbonate (69
25 mg, 0.50 mmol) were added sequentially to a stirred suspension of methyl 3-(hydroxyphenyl)-propionate (90 mg, 0.50 mmol) in dimethylformamide (3 ml). The reaction was heated at 100 °C for 4 hours then allowed to stir for a further 36 hours at ambient temperature. Brine (10 ml) was added and the reaction allowed to stand for 16 hours before the solid was collected by suction filtration (analogous reactions which failed to yield a solid precipitate were extracted
30 with dichloromethane (2 x 5 ml) and the dichloromethane layer evaporated *in vacuo* to give a

solid product). Drying *in vacuo* yielded the title compound (135 mg, 73 % yield) as a white solid :

¹H-NMR (DMSO d₆) : 8.56 (s, 1H), 7.57 (s, 1H), 7.40 (s, 1H), 7.35 (d, 2H), 7.23 (d, 2H), 4.00 (s, 3H), 3.98 (s, 3H), 3.62 (s, 3H), 2.90 (t, 2H), 2.70 (t, 2H) :

5. MS (+ve ESI) : 369 (M+H)⁺.

4-Chloro-6,7-dimethoxyquinazoline, used as the starting material was obtained as follows :

10 a) A mixture of 4,5-dimethoxyanthranilic acid (19.7g, 100 mmol) and formamide (10ml) was heated at 190 °C for 5 hours. The mixture was allowed to cool to approximately 80 °C and water (50ml) was added. The mixture was then allowed to stand at ambient temperature for 3 hours. Collection of the solid by suction filtration, followed by washing with water (2 x 50 ml) and drying *in vacuo*, yielded 6,7-dimethoxy-3,4-dihydroquinazolin-4-one (3.65g, 18 % yield) as a white solid.

¹H-NMR (DMSO d₆) : 12.10 (s, 1H), 7.95 (s, 1H), 7.42 (s, 1H), 7.11 (s, 1H), 3.88 (s, 3H), 3.84 (s, 3H) :

15 MS (-ve ESI) : 205 (M-H)⁻.

20 b) Dimethylformamide (0.2 ml) was added dropwise to a solution of 6,7-dimethoxy-3,4-dihydro-quinazolin-4-one (10.0 g, 48.5 mmol) in thionyl chloride (200ml) and the reaction was heated at reflux for 6 hours. The reaction was cooled, excess thionyl chloride was removed *in vacuo* and the residue was azeotroped with toluene (2 x 50 ml) to remove the last of the thionyl chloride. The residue was taken up in dichloromethane (550 ml), the solution was washed with saturated aqueous sodium hydrogen carbonate solution (2 x 250 ml) and the organic phase was dried over magnesium sulphate. Solvent evaporation *in vacuo* yielded 4-chloro-6,7-dimethoxyquinazoline (10.7 g, 98 % yield) as a white solid :

¹H-NMR (DMSO d₆) : 8.86 (s, 1H), 7.42 (s, 1H), 7.37 (s, 1H), 4.00 (s, 3H), 3.98 (s, 3H) :

25 MS (+ve ESI) : 225 (M-H)⁺.

Example 2 - Preparation of Compound No. 2 in Table 1

30 An analogous reaction to that described in example 1, but starting with 4-hydroxy-4'-nitrobiphenyl (108 mg, 0.50 mmol) yielded the title compound (188 mg, 93 % yield) as a white solid :

$^1\text{H-NMR}$ (DMSO d_6) : 8.58 (s, 1H), 8.33 (d, 2H), 8.02 (d, 2H), 7.92 (d, 2H), 7.59 (s, 1H), 7.49 (d, 2H), 7.40 (s, 1H), 4.00 (s, 3H), 3.99 (s, 1H) :

MS (+ve ESI) : 404 (M+H) $^+$.

5 **Example 3 - Preparation of Compound No. 3 in Table 1**

~~An analogous reaction to that described in example 1, but starting with 4-~~
phenylphenol (85 mg, 0.50 mmol) yielded the title compound (170 mg, 95 % yield) as a white solid :

10 $^1\text{H-NMR}$ (DMSO d_6) : 8.60 (s, 1H), 7.79 (d, 2H), 7.73 (d, 2H), 7.61 (s, 1H), 7.47-7.55 (m, 2H), 7.37-7.46 (m, 4H), 4.00 (s, 6H) :

MS (+ve ESI) : 359 (M+H) $^+$.

Example 4 - Preparation of Compound No. 4 in Table 1

15 An analogous reaction to that described in example 1 but starting with 4-n-propylphenol (68 mg, 0.50 mmol) yielded the title compound (87 mg, 54 % yield) as a white solid :

$^1\text{H-NMR}$ (DMSO d_6) : 8.55 (s, 1H), 7.57 (s, 1H), 7.39 (s, 1H), 7.30 (d, 2H), 7.21 (d, 2H), 4.00 (s, 3H), 3.98 (s, 3H), 2.61 (t, 2H), 1.58-1.72 (m, 2H), 0.94 (t, 3H) :

MS (+ve ESI) : 325 (M+H) $^+$.

20 **Example 5 - Preparation of Compound No. 5 in Table 1**

An analogous reaction to that described in example 1, but starting with 4-hydroxydiphenylmethane (92 mg, 0.50 mmol) yielded the title compound (44 mg, 24 % yield) as a white solid :

25 $^1\text{H-NMR}$ (DMSO d_6) : 8.53 (s, 1H), 7.55 (s, 1H), 7.38 (s, 1H), 7.28-7.37 (m, 7H), 7.23 (d, 2H), 4.01 (s, 2H), 4.00 (s, 3H), 3.98 (s, 3H) :

MS (+ve ESI) : 373 (M+H) $^+$.

Example 6 - Preparation of Compound No. 6 in Table 1

An analogous reaction to that described in example 1, but starting with 4-bromo-4'-hydroxybiphenyl (125 mg, 0.50 mmol) yielded the title compound (205 mg, 94 % yield) as a white solid :

5 ¹H-NMR (DMSO d₆) : 8.59 (s, 1H), 7.79 (d, 2H), 7.69 (s, 4H), 7.60 (s, 1H), 7.42 (d, 2H), 7.40 (s, 1H), 4.01 (s, 3H), 4.00 (s, 3H) :

MS (+ve ESI) : 437 (M+H)⁺.

Example 7 - Preparation of Compound No7 in Table 1

10 An analogous reaction to that described in example 1, but starting with 3-(4-hydroxyphenyl)propionitrile (73 mg, 0.50 mmol) yielded the title compound (150 mg, 89 % yield) as a white solid :

¹H-NMR (DMSO d₆) : 8.57 (s, 1H), 7.57 (s, 1H), 7.41 (d, 2H), 7.39 (s, 1H), 7.28 (d, 2H), 4.00 (s, 3H), 3.99 (s, 3H), 2.82-3.00 (m, 4H) :

15 MS (+ve ESI) : 336 (M+H)⁺.

Example 8 - Preparation of Compound No. 8 in Table 1

Bis(triphenylphosphine) palladium (II) chloride (570 mg, 0.81 mmol) was added to a mixture of triethylamine (2.38 ml, 41.2 mmol), 4-(4-iodoanilino)-6,7-dimethoxyquinazoline (3.00 g, 6.77 mmol), copper (I) iodide (154 mg, 0.81 mmol) and (trimethylsilyl)acetylene (2.66 ml, 20.3 mmol) in tetrahydrofuran (60 ml) under an inert atmosphere and the reaction was stirred for 48 hours at ambient temperature. The solvents were removed *in vacuo*, the reaction was partitioned between ethyl acetate (50 ml) and water (50 ml) and the biphasic mixture was filtered through celite. The organic layer was separated, washed with brine (50 ml), dried over magnesium sulphate and evaporated *in vacuo*. Purification by flash chromatography on silica gel, eluting with 50-100% ethyl acetate in isohexane, yielded the title compound (1.95 g, 77 % yield) as a yellow solid :

25 ¹H-NMR (DMSO d₆) : 9.30 (s, 1H), 8.31 (s, 1H), 7.70 (d, 2H), 7.60 (s, 1H), 7.25 (d, 2H), 7.01 (s, 1H), 3.75 (s, 3H), 3.70 (s, 3H), 0.00 (s, 9H) :

30 MS (-ve ESI) : 376 (M-H)⁻,

MS (+ve ESI) : 378 (M+H)⁺.

4-(4-iodoanilino)-6,7-dimethoxyquinazoline, used as the starting material was obtained as follows :

A solution of 4-iodoaniline (4.89 g, 22.3 mmol) and 4-chloro-6,7-dimethoxyquinazoline (5.00 g, 22.3 mmol), in isopropanol (200 ml) was heated at reflux for 3 hours before the reaction was allowed to cool to ambient temperature. The solid which had precipitated was collected by suction filtration and washed with diethyl ether (2 x 50 ml). Drying of this material yielded 4-(4-iodoanilino)-6,7-dimethoxyquinazoline (9.38 g, 95 % yield) as a white solid :

¹H-NMR (DMSO d₆) : 11.33 (s, 1H), 8.81 (s, 1H), 8.30 (s, 1H), 7.80 (s, 1H), 7.55 (d, 2H), 7.30 (s, 1H), 4.02 (s, 3H), 3.93 (s, 3H) :

MS (-ve ESI) : 406 (M-H)⁻,

MS (+ve ESI) : 408 (M+H)⁺.

Example 9 - Preparation of Compound No. 9 in Table 1

Tetrabutylammonium fluoride (5.84 ml of a 1.0N solution in tetrahydrofuran, 5.84 mmol) was added to a solution of 4-(4-(2-(trimethylsilyl)ethynyl)anilino)-6,7-dimethoxyquinazoline (1.83 g, 4.85 mmol) in tetrahydrofuran (150 ml) at 10 °C under an inert atmosphere and the reaction allowed to stir for 10 minutes at 10 °C before the reaction was poured into brine (100 ml). The reaction was extracted with ethyl acetate (3 x 50 ml), the combined organic layers were washed with saturated aqueous sodium bicarbonate solution (100 ml), dried over magnesium sulphate and evaporated *in vacuo*. Purification by flash chromatography on silica gel, eluting with i) 50-100% ethyl acetate in isohexane ii) 10% methanol in ethyl acetate, yielded the title compound (0.54 g, 36 % yield) as a yellow solid :

¹H-NMR (DMSO d₆) : 9.55 (s, 1H), 8.51 (s, 1H), 7.90 (d, 2H), 7.81 (s, 1H), 7.45 (d, 2H), 7.20 (s, 1H), 4.05 (s, 1H), 4.00 (s, 3H), 3.95 (s, 3H) :

MS (-ve ESI) : 304 (M-H)⁻,

MS (+ve ESI) : 306 (M+H)⁺.

Example 10 - Preparation of Compound No. 10 in Table 1

A slurry of 10% palladium on carbon (20 mg) in acetic acid (3 ml) was added to a solution of 4-(4-(2-carboethoxy)ethenyl)anilino)-6,7-dimethoxyquinazoline (200 mg, 0.53 mmol) and the reaction was stirred for 48 hours at ambient temperature under an atmosphere of hydrogen. The reaction was filtered through celite, the solvents were removed *in vacuo* and the residue was treated with aqueous sodium hydrogen carbonate solution (50 ml). The reaction was extracted with a mixture of ethyl acetate (25 ml) and diethyl ether (25 ml) and the combined organic layers were evaporated *in vacuo* to yield 4-(4-(2-carboethoxy)ethyl)anilino)-6,7-dimethoxyquinazoline (165 mg, 82 % yield) as a white solid :

¹H-NMR (DMSO *d*₆) : 9.40 (s, 1H), 8.42 (s, 1H), 7.79 (s, 1H), 7.65 (d, 2H), 7.20 (d, 2H), 7.19 (s, 1H), 4.00-4.10 (m, 2H), 3.95 (s, 3H), 3.93 (s, 3H), 2.79-2.90 (m, 2H), 2.60-2.65 (m, 2H), 1.10-1.20 (m, 2H) :

MS (-ve ESI) : 380 (M-H)⁻,

MS (+ve ESI) : 382 (M+H)⁺.

Example 11 - Preparation of Compound No. 11 in Table 1

A solution of 1-(4-aminophenyl)phenylacetonitrile (41 mg, 0.20 mmol) and 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline (67.5 mg, 0.20 mmol), in isopropanol (10 ml) was heated at reflux for 3.5 hours before the reaction was allowed to cool to ambient temperature. The solid which had precipitated was collected by suction filtration and washed with diethyl ether (2 x 10 ml). Drying of this material yielded the title compound (96 mg, 80 % yield) as a white solid :

¹H-NMR (DMSO *d*₆) : 11.27 (s, 1H), 8.75 (s, 1H), 8.31 (s, 1H), 7.74 (d, 2H), 7.49 (d, 2H), 7.29-7.46 (m, 6H), 5.87 (s, 1H), 4.30 (t, 2H), 4.01 (s, 3H), 3.71-4.01 (m, 4H), 3.00-3.57 (m, 6H), 2.23-2.39 (m, 2H) ;

MS (+ve ESI) : 510 (M+H)⁺.

4-Chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline, used as the starting material, was obtained as follows :

a) A mixture of morpholine (261 ml, 3.00 mol) and 1-bromo-3-chloropropane (148 ml, 1.50 mol) in toluene (900 ml) was stirred for 18 hours at ambient temperature. Additional 1-

bromo-3-chloropropane (25 ml, 0.25 mol) was added, the reaction was stirred for a further 1 hour and then filtered to remove the precipitated solid before the filtrate was concentrated *in vacuo*. Distillation of the crude oil yielded N-(3-chloropropyl)-morpholine (119.3 g, 49 % yield) as the fraction boiling at 70 - 80 °C / 2.6 mmHg :

5 ¹H-NMR (DMSO d₆) : 3.65 (t, 2H), 3.55 (m, 4H), 2.4 (t, 2H), 2.39 (m, 4H), 1.85 (m, 2H) :
MS (+ve ESI) : 164 (M+H)⁺.

b) N-(3-Chloropropyl)morpholine (90 g, 0.55 mol) was added dropwise, over 30 minutes, to a solution of ethyl vanillate (98 g, 0.50 mol) and powdered potassium carbonate (104 g, 0.75 mol) in dimethylformamide (300 ml) at 80 °C. The reaction was heated at 80 °C for 90
10 minutes, cooled to ambient temperature, filtered and the filtrate concentrated *in vacuo*. The crude product was taken up in diethyl ether (1000 ml), filtered and washed with water (2 x 200 ml) and brine (200 ml). Solvent evaporation in vacuo yielded ethyl 3-methoxy-4-(3-morpholinopropoxy)benzoate (161.5 g, 100 % yield) as a pale yellow oil which crystallised on standing to afford a pale yellow solid :

15 ¹H-NMR (DMSO d₆) : 7.55 (dd, 1H), 7.4 (d, 1H), 7.05 (d, 1H), 4.3 (q, 2H), 4.05 (t, 2H), 3.8 (s, 3H), 3.55 (m, 4H), 2.4 (t, 2H), 2.35 (m, 4H), 1.9 (m, 2H), 1.3 (t, 3H) :
MS (-ve ESI) : 324 (M-H)⁻,

c) Concentrated sulphuric acid (110 ml) and concentrated nitric acid (19.0 ml, 0.289 mol) were added cautiously, over a 50 minute period, to a two-phase system containing a stirred
20 solution of ethyl 3-methoxy-4-(3-morpholinopropoxy)benzoate (76.5 g, 0.237 mol) in dichloromethane (600 ml), acetic acid (300 ml) and water (70 ml) at 5 °C. The reaction was allowed to warm to ambient temperature over 18 hours, the aqueous phase was separated, and the aqueous phase was taken to pH 9 by addition of 40% aqueous sodium hydroxide solution (775 ml). Extraction of the aqueous phase with dichloromethane (3 x 600 ml) and subsequent
25 solvent evaporation *in vacuo* yielded ethyl 3-methoxy-4-(3-morpholinopropoxy)-6-nitrobenzoate (141.3 g, 86 % yield) as a yellow gum :

¹H-NMR (CDCl₃) : 7.5 (s, 1H), 7.1 (s, 1H), 4.4 (q, 2H), 4.2 (t, 2H), 4.0 (s, 3H), 3.7 (m, 4H), 2.5 (t, 2H), 2.45 (m, 4H), 2.05 (m, 2H), 1.4 (t, 3H) :
MS (+ve ESI) : 369 (M+H)⁺.

30 d) A suspension of ethyl 3-methoxy-4-(3-morpholinopropoxy)-6-nitrobenzoate (132.2 g, 359 mmol) and 10% palladium on carbon (3.0 g) in a mixture of ethanol (200 ml) and ethyl

acetate (2000 ml) was stirred under an atmosphere of hydrogen for 18 hours. Removal of the catalyst by filtration, followed by solvent evaporation *in vacuo* yielded ethyl 3-methoxy-4-(3-morpholinopropoxy)-6-aminobenzoate (122 g, 100 % yield) as a brown oil :

¹H-NMR (DMSO *d*₆) : 7.15 (s, 1H), 6.4 (s, 2H), 6.35 (s, 1H), 4.2 (q, 2H), 3.95 (t, 2H), 3.65 (s, 3H), 3.55 (m, 4H), 2.4 (t, 2H), 2.35 (m, 4H), 1.85 (m, 2H), 1.25 (t, 3H) :

MS (-ve ESI) : 337 (M-H),

MS (+ve ESI) : 339 (M+H)⁺.

e) A solution of ethyl 3-methoxy-4-(3-morpholinopropoxy)-6-aminobenzoate (130 g, 384 mmol) in formamide (280 ml) was heated at 180 °C for 3 hours, during which time a small amount (25 ml) of liquid distilled out of the reaction. The reaction was cooled to 125 °C and the excess formamide was evaporated *in vacuo*. Trituration of the solid residue with isopropanol (100 ml), followed by drying *in vacuo*, yielded 6-methoxy-7-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (83.0 g, 68 % yield) as a pale brown solid :

¹H-NMR (DMSO *d*₆) : 12.0 (s, 1H), 7.95 (s, 1H), 7.45 (s, 1H), 7.1 (s, 1H), 4.15 (t, 2H), 3.85 (s, 3H), 3.6 (m, 4H), 2.45 (t, 2H), 2.35 (m, 4H), 1.9 (m, 2H) :

MS (-ve ESI) : 318 (M-H),

MS (+ve ESI) : 320 (M+H)⁺.

f) Dimethylformamide (2.0 ml) was added dropwise to a solution of 6-methoxy-7-(3-morpholinopropoxy)-3,4-dihydro-quinazolin-4-one (83.0 g, 261 mmol) in thionyl chloride (700ml) and the reaction was heated at reflux for 3.5 hours. The reaction was cooled, excess thionyl chloride was removed *in vacuo*, the residue was taken up in water (500 ml) and this aqueous solution was taken to pH 9 by addition of saturated aqueous sodium bicarbonate solution (300 ml). The aqueous phase was extracted with dichloromethane (2 x 400 ml), the organic solution was washed with brine (400 ml) and the solvents were removed *in vacuo*.

Trituration of the solid residue with ethyl acetate (150 ml), followed by drying *in vacuo*, yielded 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline (53 g, 60 % yield) as a pale brown solid :

¹H-NMR (CDCl₃) : 8.85 (s, 1H), 7.39 (s, 1H), 7.38 (s, 1H), 4.3 (t, 2H), 4.05 (s, 3H), 3.7 (m, 4H), 2.6 (t, 2H), 2.5 (m, 4H), 2.1 (m, 2H) :

MS (+ve ESI) : 338 (M+H)⁺.

Example 12 - Preparation of Compound No. 12 in Table 1

A solution of 1.0N hydrochloric acid in ether (0.50 ml, 0.50 mmol) was added to a solution of 4-hexylaniline (89 mg, 0.50 mmol) and 4-chloro-6-methoxy-7-(3-morpholinopropoxy)quinazoline (168 mg, 0.50 mmol), in isopropanol (5.0 ml). The reaction was heated at 40 °C for 30 minutes and then at 83 °C for 12 hours. The reaction was allowed to cool to ambient temperature and the solid which had precipitated was collected by suction filtration and washed with diethyl ether (2 x 10 ml). Drying of this material yielded the title compound (173 mg, 67 % yield) as a white solid :

¹H-NMR (DMSO d₆) : 11.43 (s, 1H), 11.18 (s, 1H), 8.78 (s, 1H), 8.38 (s, 1H), 7.60 (d, 2H), 7.43 (s, 1H), 7.30 (d, 2H), 4.32 (t, 2H), 4.03 (s, 3H), 3.75-4.03 (m, 4H), 3.00-3.60 (m, 6H), 2.62 (t, 2H), 2.28-2.42 (m, 2H), 1.53-1.68 (m, 2H), 1.21-1.40 (m, 6H); 0.88 (t, 3H) ;
MS (+ve ESI) : 479 (M+H)⁺.

Example 13 - Preparation of Compound No. 13 in Table 1

An analogous reaction to that described in example 12, but starting with 4-n-butylaniline (75 mg, 0.50 mmol) yielded the title compound (168 mg, 69 % yield) as a white solid :

¹H-NMR (DMSO d₆) : 11.40 (s, 1H), 11.15 (s, 1H), 8.80 (s, 1H), 8.40 (s, 1H), 7.60 (d, 2H), 7.42 (s, 1H), 7.3 (d, 2H), 4.32 (t, 2H), 4.04 (s, 3H), 3.95 (m, 2H), 3.85 (m, 2H), 3.50 (m, 2H), 3.35 (m, 2H), 3.10 (m, 2H), 2.65 (m, 2H), 2.35 (m, 2H), 1.60 (m, 2H), 1.35 (m, 2H), 0.91 (t, 3H) ;
MS (+ve ESI) : 451 (M+H)⁺.

Example 14 - Preparation of Compound No. 14 in Table 1

An analogous reaction to that described in example 12, but starting 4-aminodiphenylmethane (92 mg, 0.50 mmol) yielded the title compound (235 mg, 90 % yield) as a white solid :

¹H-NMR (DMSO d₆) : 11.35 (s, 1H), 11.10 (s, 1H), 8.75 (s, 1H), 8.35 (s, 1H), 7.60 (d, 2H), 7.35 (s, 1H), 7.30 (m, 6H), 7.20 (t, 1H), 4.30 (t, 2H), 4.00 (s, 3H), 3.97 (s, 2H), 3.95 (m, 2H), 3.84 (m, 2H), 3.50 (m, 2H), 3.30 (m, 2H), 3.10 (m, 2H), 2.35 (m, 2H) ;
MS (+ve ESI) : 485 (M+H)⁺.

Biological Data

The compounds of the invention inhibit the serine/threonine kinase activity of the aurora2 kinase and thus inhibit the cell cycle and cell proliferation. These properties may be assessed, for example, using one or more of the procedures set out below:

5

(a) In Vitro aurora2 kinase inhibition test

This assay determines the ability of a test compound to inhibit serine/threonine kinase activity. DNA encoding aurora2 may be obtained by total gene synthesis or by cloning. This DNA may then be expressed in a suitable expression system to obtain polypeptide with serine/threonine kinase activity. In the case of aurora2, the coding sequence was isolated from cDNA by polymerase chain reaction (PCR) and cloned into the BamH1 and Not1 restriction endonuclease sites of the baculovirus expression vector pFastBac HTc (GibcoBRL/Life technologies). The 5' PCR primer contained a recognition sequence for the restriction endonuclease BamH1 5' to the aurora2 coding sequence. This allowed the insertion of the aurora2 gene in frame with the 6 histidine residues, spacer region and rTEV protease cleavage site encoded by the pFastBac HTc vector. The 3' PCR primer replaced the aurora2 stop codon with additional coding sequence followed by a stop codon and a recognition sequence for the restriction endonuclease Not1. This additional coding sequence (5' TAC CCA TAC GAT GTT CCA GAT TAC GCT TCT TAA 3') encoded for the polypeptide sequence YPYDVDPDYAS. This sequence, derived from the influenza hemagglutinin protein, is frequently used as a tag epitope sequence that can be identified using specific monoclonal antibodies. The recombinant pFastBac vector therefore encoded for an N-terminally 6 his tagged, C terminally influenza hemagglutinin epitope tagged aurora2 protein. Details of the methods for the assembly of recombinant DNA molecules can be found in standard texts, for example Sambrook et al. 1989, Molecular Cloning - A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press and Ausubel et al. 1999, Current Protocols in Molecular Biology, John Wiley and Sons Inc.

Production of recombinant virus can be performed following manufacturer's protocol from GibcoBRL. Briefly, the pFastBac-1 vector carrying the aurora2 gene was transformed into E. coli DH10Bac cells containing the baculovirus genome (bacmid DNA) and via a transposition

event in the cells, a region of the pFastBac vector containing gentamycin resistance gene and the aurora2 gene including the baculovirus polyhedrin promoter was transposed directly into the bacmid DNA. By selection on gentamycin, kanamycin, tetracycline and X-gal, resultant white colonies should contain recombinant bacmid DNA encoding aurora2. Bacmid DNA was
5 extracted from a small scale culture of several BH10Bac white colonies and transfected into ~~Spodoptera frugiperda Sf21 cells grown in TC100 medium (GibcoBRL) containing 10%~~

serum using CellFECTIN reagent (GibcoBRL) following manufacturer's instructions. Virus particles were harvested by collecting cell culture medium 72 hrs post transfection. 0.5 mls of medium was used to infect 100 ml suspension culture of Sf21s containing 1×10^7 cells/ml.
10 Cell culture medium was harvested 48 hrs post infection and virus titre determined using a standard plaque assay procedure. Virus stocks were used to infect Sf9 and "High 5" cells at a multiplicity of infection (MOI) of 3 to ascertain expression of recombinant aurora2 protein.

For the large scale expression of aurora2 kinase activity, Sf21 insect cells were grown at 28°C in TC100 medium supplemented with 10% foetal calf serum (Viralex) and 0.2% F68
15 Pluronic (Sigma) on a Wheaton roller rig at 3 r.p.m. When the cell density reached 1.2×10^6 cells ml^{-1} they were infected with plaque-pure aurora2 recombinant virus at a multiplicity of infection of 1 and harvested 48 hours later. All subsequent purification steps were performed at 4°C. Frozen insect cell pellets containing a total of 2.0×10^8 cells were thawed and diluted with lysis buffer (25 mM HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulphonic
20 acid]) pH7.4 at 4°C, 100 mM KCl, 25 mM NaF, 1 mM Na_3VO_4 , 1 mM PMSF (phenylmethylsulphonyl fluoride), 2 mM 2-mercaptoethanol, 2 mM imidazole, 1 µg/ml aprotinin, 1 µg/ml pepstatin, 1 µg/ml leupeptin), using 1.0 ml per 3×10^7 cells. Lysis was achieved using a dounce homogeniser, following which the lysate was centrifuged at 41,000g for 35 minutes. Aspirated supernatant was pumped onto a 5 mm diameter chromatography
25 column containing 500 µl Ni NTA (nitrilo-tri-acetic acid) agarose (Qiagen, product no. 30250) which had been equilibrated in lysis buffer. A baseline level of UV absorbance for the eluent was reached after washing the column with 12 ml of lysis buffer followed by 7 ml of wash buffer (25 mM HEPES pH7.4 at 4°C, 100 mM KCl, 20 mM imidazole, 2 mM 2-mercaptoethanol). Bound aurora2 protein was eluted from the column using elution buffer (25
30 mM HEPES pH7.4 at 4°C, 100 mM KCl, 400 mM imidazole, 2 mM 2-mercaptoethanol). An elution fraction (2.5 ml) corresponding to the peak in UV absorbance was collected. The

elution fraction, containing active aurora2 kinase, was dialysed exhaustively against dialysis buffer (25 mM HEPES pH7.4 at 4°C , 45% glycerol (v/v), 100 mM KCl, 0.25% Nonidet P40 (v/v), 1 mM dithiothreitol).

Each new batch of aurora2 enzyme was titrated in the assay by dilution with enzyme diluent (25mM Tris-HCl pH7.5, 12.5mM KCl, 0.6mM DTT). For a typical batch, stock enzyme is diluted 1 in 666 with enzyme diluent & 20µl of dilute enzyme is used for each assay well. Test compounds (at 10mM in dimethylsulphoxide (DMSO)) were diluted with water & 10µl of diluted compound was transferred to wells in the assay plates. "Total" & "blank" control wells contained 2.5% DMSO instead of compound. Twenty microlitres of freshly diluted enzyme was added to all wells, apart from "blank" wells. Twenty microlitres of enzyme diluent was added to "blank" wells. Twenty microlitres of reaction mix (25mM Tris-HCl, 78.4mM KCl, 2.5mM NaF, 0.6mM dithiothreitol, 6.25mM MnCl₂, 6.25mM ATP, 7.5µM peptide substrate [biotin-LRRWSLGLRRWSLGLRRWSLGLRRWSLGLG]) containing 0.2µCi [γ ³³P]ATP (Amersham Pharmacia, specific activity ≥ 2500 Ci/mmol) was then added to all test wells to start the reaction. The plates were incubated at room temperature for 60 minutes. To stop the reaction 100µl 20% v/v orthophosphoric acid was added to all wells. The peptide substrate was captured on positively-charged nitrocellulose P30 filtermat (Whatman) using a 96-well plate harvester (TomTek) & then assayed for incorporation of ³³P with a Beta plate counter. "Blank" (no enzyme) and "total" (no compound) control values were used to determine the dilution range of test compound which gave 50% inhibition of enzyme activity.

In this test, compound 11 in Table 1 gave 50% inhibition of enzyme activity at a concentration of 0.279µM.

(b) In Vitro cell proliferation assay

This assay determines the ability of a test compound to inhibit the growth of adherent mammalian cell lines, for example the human tumour cell line MCF7.

MCF-7 (ATCC HTB-22) or other adherent cells were typically seeded at 1×10^3 cells per well (excluding the peripheral wells) in DMEM (Sigma Aldrich) without phenol red, plus 10% foetal calf serum, 1% L-glutamine and 1% penicillin/streptomycin in 96 well tissue culture treated clear plates (Costar). The following day (day 1), the media was removed from a no treatment control plate and the plate stored at -80°C. The remaining plates were dosed

with compound (diluted from 10mM stock in DMSO using DMEM (without phenol red, 10% FCS, 1% L-glutamine, 1% penicillin/streptomycin). Untreated control wells were included on each plate. After 3 days in the presence / absence of compound (day 4) the media was removed and the plates stored at -80°C. Twenty four hours later the plates were thawed at room temperature and cell density determined using the CyQUANT cell proliferation assay kit (c-7026/c-7027 Molecular Probes Inc.) according to manufacturers directions. Briefly, 200µl of a cell lysis / dye mixture (10µl of 20X cell lysis buffer B, 190µl of sterile water, 0.25µl of CYQUANT GR dye) was added to each well and the plates incubated at room temperature for 5 minutes in the dark. The fluorescence of the wells was then measured using a fluorescence microplate reader (gain 70, 2 reads per well, 1 cycle with excitation 485nm and emission 530nm using a CytoFluor plate reader (PerSeptive Biosystems Inc.)). The values from day 1 and day 4 (compound treated) together with the values from the untreated cells were used to determine the dilution range of a test compound that gave 50% inhibition of cell proliferation. Compound 11 in Table 1 was effective in this test at 3.24µM.

These values could also be used to calculate the dilution range of a test compound at which the cell density dropped below the day 1 control value. This indicates the cytotoxicity of the compound.

(c) In Vitro cell cycle analysis assay

This assay determines the ability of a test compound to arrest cells in specific phases of the cell cycle. Many different mammalian cell lines could be used in this assay and MCF7 cells are included here as an example. MCF-7 cells were seeded at 3×10^5 cells per T25 flask (Costar) in 5 ml DMEM (no phenol red 10% FCS, 1% L-glutamine 1% penicillin / streptomycin). Flasks were then incubated overnight in a humidified 37°C incubator with 5% CO₂. The following day 1ml of DMEM (no phenol red 10% FCS, 1% L-glutamine 1% penicillin / streptomycin) carrying the appropriate concentration of test compound solubilised in DMSO was added to the flask. A no compound control treatments was also included (0.5% DMSO). The cells were then incubated for a defined time (usually 24 hours) with compound. After this time the media was aspirated from the cells and they were washed with 5ml of prewarmed (37°C) sterile PBSA, then detached from the flask by brief incubation with trypsin

and followed by resuspension in 10ml of 1% Bovine Serum Albumin (BSA, Sigma-Aldrich Co.) in sterile PBSA. The samples were then centrifuged at 2200rpm for 10 min. The supernatant was aspirated and the cell pellet was resuspended in 200 μ l of 0.1% (w/v) Tris sodium citrate, 0.0564% (w/v) NaCl, 0.03% (v/v) Nonidet NP40, [pH 7.6]. Propidium Iodide (Sigma Aldrich Co.) was added to 40 μ g/ml and RNAase A (Sigma Aldrich Co.) to 100 μ g/ml.

The cells were then incubated at 37°C for 30 minutes. The samples were centrifuged at 2200rpm for 10 min, the supernatant removed and the remaining pellet (nuclei) resuspended in 200 μ l of sterile PBSA. Each sample was then syringed 10 times using 21 gauge needle. The samples were then transferred to LPS tubes and DNA content per cell analysed by Fluorescence activated cell sorting (FACS) using a FACScan flow cytometer (Becton Dickinson). Typically 25000 events were counted and recorded using CellQuest v1.1 software (Verity Software). Cell cycle distribution of the population was calculated using Modfit software (Verity Software) and expressed as percentage of cells in G0/G1, S and G2/M phases of the cell cycle.

Treating MCF7 cells with 3.2 μ M Compound 11 in Table 1 for 24 hours produced the following changes in cell cycle distribution:

Treatment	% Cells in G1	% Cells in S	% Cells in G2/M
DMSO (control)	49.96	46	4.4
3.2 μ M Compound 11	19.85	12.13	68.01

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